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(54) **Improved formulation for recombinant beta-interferon processes for recovery and stabilization of beta-interferon and the use thereof.**

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A total of 3.33 ml of the desalted IFN-β having 0.25 mg IFN per ml was used in each experiment and the pH of each mixture was adjusted to 3 before incubation and adjusted to between 7.3 and 7.5 after incubation. The results are indicated in the table below.

Stabilizer	Amount of Stabilizer (%)	Incubation Time (min.)	Clarity at pH 7.3-7.5
HSA	2.5	45	slightly hazy
HSA	5.0	45	very clear
HSA	2.5	15	slightly hazy
HSA	5.0	15	very clear
PPF	2.5	45	very clear
PPF	2.5	15	very clear

The PPF formulations were found, when examined visually, to have the best clarity, with 5.0% HSA next best, followed by 2.5% HSA. When each formulation was lyophilized and reconstituted with water, the PPF formulations reconstituted more clearly than the HSA formulations. All lyophilized formulations had IFN-β activity.

Experiments done at pH 3-4 without an incubation period appeared to show no appreciable difference from those that undergo an incubation period. Changes in concentration of PPF do result, however, in a marked difference in clarity.

Experiments to optimize the pH 3 formulation revealed that 5% HSA was distinctly better than the 2.5% HSA formulation. Also, increasing the incubation time from 15 minutes to 60 minutes assisted the solubility. The 5% HSA at 15 minutes incubation, however, was found to be better than the 2.5% HSA at 60 minutes incubation.

The above results indicate that for IFN-β, PPF is a better stabilizer than HSA. In addition, tests for biological activity of representative formulated proteins at pH 3 revealed that the IFN-β was biologically active.

III. Low pH Mannitol Formulations

Stabilizers can be used to stabilize recombinant beta-interferon formulations at a low pH of 2-4 if the pH is maintained at 2-4 and lyophilized at pH 2-4. If the pH is raised above 4, however, the carbohydrate stabilizers such as mannitol will not act to solubilize the lipophilic protein. Only the protein stabilizers such as HSA will solubilize the protein as the pH is raised to physiological pH.

Conclusion

The process and compositions of the present invention as described herein yield a recombinant β-HIFN preparation which is of relatively high purity, with residual SDS levels of less than about 10 p.p.m. and which may be formulated into therapeutically acceptable preparations in a non-toxic, inert, physiologically compatible carrier medium for clinical and therapeutic uses. The principal advantage of the instant invention lies in the reduction of SDS levels in the protein preparation (which can potentiate hepatic toxicity in some patients) to about 2-20 p.p.m., preferably to less than about 10 p.p.m., and more preferably to about 2-6 p.p.m., which are therapeutically acceptable.

Claims

1. A stable pharmaceutical composition comprising a therapeutically effective amount of a biologically active recombinant β-HIFN dissolved in a non-toxic, inert, therapeutically compatible aqueous-based carrier medium at a pH of 2 to 4 and a stabilizer selected from human serum albumin, human plasma protein fraction, mannitol, sorbitol, glycerol, dextrose, or a mixture thereof.

2. A composition according to claim 1 wherein the stabilizer is human plasma protein fraction and is present in an amount of 0.1 to 5% (w/v) or the stabilizer is human serum albumin and is present in a concentration range of about 0.5 to 10% (w/v).

3. A composition according to claim 1 or claim 2 wherein any sodium dodecyl sulfate present is at a level of less than 10 p.p.m.

4. A composition according to any one of claims 1 to 3 which is lyophilized.

5. A process for reconstituting into a therapeutically acceptable formulation a substantially surfactant free recombinant β-HIFN obtainable from a host transformed to produce it wherein the cell wall of the host is disrupted and the protein in the disruptate is isolated and purified, comprising the steps of:
(a) adjusting the pH of the medium in which the β-HIFN is contained to about 2 to 4;
(b) adding to the β-HIFN medium a stabilizer for the β-HIFN which has been previously adjusted to a pH of about 2 to 4; and
(c) lyophilizing the resulting composition at about pH 2 to 4.

6. The process of claim 5 wherein the stabilizer is mannitol.

7. A process for reconstituting into a therapeutically acceptable formulation a substantially surfactant free recombinant β-HIFN obtainable from a host transformed to produce it wherein the cell wall of the host is disrupted and the protein in the disruptate is isolated and purified, comprising the steps of:
(a) adjusting the pH of the medium in which the β-HIFN is contained to about 2 to 4;
(b) adding to the β-HIFN medium a protein stabilizer for the β-HIFN protein which has been previously adjusted to a pH of about 2 to 4; and
(c) raising the pH of the resulting composition to 6.8-7.8.

8. A process for reconstituting into a therapeutically acceptable formulation a substantially surfactant free recombinant β-HIFN obtainable from a host transformed to produce it wherein the cell wall of the host is disrupted and the β-HIFN is isolated and purified, comprising:
(a) combining the β-HIFN with a protein stabilizer and adjusting the pH of the combination to about 2 to 4; and
(b) raising the pH of the resulting composition to 6.8 to 7.8;
the β-HIFN optionally being desalted at a pH of 9.2-11 by chromatography just prior to step (a).

9. A process for stabilising a biologically active recombinant β-HIFN comprising dissolving the β-HIFN in a non-toxic, inert, therapeutically compatible aqueous-based carrier medium at a pH of 2 to 4 comprising a stabilizer selected from human serum albumin, human plasma protein fraction, mannitol, sorbitol, glycerol, dextrose, or a mixture thereof.

10. The use of a biologically active β-HIFN which has been stabilized by a process as defined in claim 8 in preparing a medicament.

Patentansprüche

1. Stabiles Arzneimittel, umfassend eine therapeutisch wirksame Menge eines biologisch aktiven rekombinanten β-HIFNs, gelöst in einem nicht-toxischen, inerten, therapeutisch verträglichen, Trägermedium auf Wasserbasis bei einem pH-Wert von 2 bis 4, und ein Stabilisierungsmittel, ausgewählt aus menschlichem Serumalbumin, menschlicher Plasmaproteinfraktion, Mannit, Sorbit, Glycerin, Dextrose oder einem Gemisch davon.

2. Mittel nach Anspruch 1, wobei das Stabilisierungsmittel menschliche Plasmaproteinfraktion ist und in einer Menge von 0,1 bis 5 % (w/v) vorliegt oder das Stabilisierungsmittel menschliches Serumalbumin ist und in einem Konzentrationsbereich von etwa 0,5 bis 10 % (w/v) vorliegt.

3. Mittel nach Anspruch 1 oder 2, wobei gegebenenfalls vorhandenes Natriumdodecylsulfat in einer Menge von weniger als 10 ppm vorliegt.

4. Mittel nach einem der Ansprüche 1 bis 3, das gefriergetrocknet ist.

5. Verfahren zum Zubereiten eines im wesentlichen von grenzflächenaktiven Mitteln freien rekombinanten β-HIFNs, erhältlich aus einem zu seiner Produktion transformierten Wirt für eine therapeutisch verträgli-